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SHORT REPORTS

THE AETIOLOGY OF TURKEY 'X' DISEASE

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Key Word Index—*Aspergillus flavus*; mycotoxins; cyclopiazonic acid; turkey 'X' disease.

Abstract—From a 1960s sample of groundnut cake, which had been implicated in the first record of turkey 'X' disease, cyclopiazonic acid has been detected at a level of 31 $\mu g\,kg^{-1}$. The presence of this mycotoxin, along with aflatoxins, explains the toxic symptoms originally recorded after the groundnut cake had been ingested by turkeys.

INTRODUCTION

Mycotoxins are secondary fungal metabolites which, if ingested, can lead to a wide range of disease conditions. The aflatoxins, produced by *Aspergillus flavus* in groundnut cake, were the mycotoxins held responsible for the outbreak of turkey 'X' disease in poultry [1]. However, the clinical observations of catarrhal or haemorrhagic enteritis and opisthotonus (characteristic posture on death), originally reported, were not characteristic of aflatoxicosis. They suggested the additional presence of cyclopiazonic acid [2], a mycotoxin produced by both aflatoxigenic and non-aflatoxigenic *A. flavus* isolates. An original sample of groundnut cake from the 1960s, associated with the first record of turkey 'X' disease, has been evaluated for the possible presence of cyclopiazonic acid.

RESULTS AND DISCUSSION

Cyclopiazonic acid, at a level of 31 $\mu g\,kg^{-1}$, was detected in a sample of groundnut cake which had been implicated in the original report of turkey 'X' disease. Cyclopiazonic acid is known to be acutely toxic to many animal species, and can accumulate to significant levels in animal tissues [3]. Furthermore, the presence of aflatoxin may enhance the accumulation of cyclopiazonic acid in poultry tissue [4]. The latter compound has also been implicated in the human kodua poisoning associated with the consumption of contaminated millet [5]. The finding of cyclopiazonic acid in the groundnut cake would explain the observation of catarrhal and haemorrhagic enteritis and opisthotonus in turkeys which had ingested the cake. Thus, the presence of cyclopiazonic acid and aflatoxins would explain more fully the toxic effects recorded. It is our opinion that the conditions under which the groundnut cake had been stored would have precluded the production of cyclopiazonic acid during the 30 years storage period.

It is important that more work is undertaken to establish the occurrence and toxicity of cyclopiazonic acid in foods and feeds, especially since the cyclopiazonic acid-producing *A. flavus* isolates significantly outnumber those producing aflatoxin.

EXPERIMENTAL

Groundnut cake responsible for the first record of turkey 'X' disease has been stored at ambient temp., in carefully sealed containers, at the Natural Resources Institute; the moisture content of the cake prior to analysis was 11%.

A sample was extracted with MeOH and the cyclopiazonic acid level determined, following a phenol/diol bonded phase column clean-up procedure, using high performance TLC and absorption densitometric quantification, at 282 nm, of the Ehrlich reagent derivative [6].

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8,16-DIHYDROXYHEXADECANOIC ACID, A MAJOR COMPONENT FROM CUCUMBER CUTIN

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Key Word Index—*Cucumis sativus*; Cucurbitaceae; cutin; hydroxy fatty acids; evaporative light-scattering detector; 8,16-dihydroxyhexadecanoic acid.

Abstract—The major component of cucumber (*Cucumis sativus*) cutin has been identified as 8,16-dihydroxyhexadecanoic acid by mass and NMR spectroscopy. This is the first report of this monomer as a major cutin component.

INTRODUCTION

We have recently described a HPLC system equipped with an evaporative light-scattering detector for the separation of underivatized hydroxy, epoxy and epoxy-hydroxy fatty acids [1]. This technique was used to analyse cutin monomers, which are mainly composed of hydroxy fatty acids [2], from various fruits e.g. grapefruit (*Citrus paradisi*), pumpkin (*Cucurbita pepo*) and green pepper (*Capsicum annuum*) [3]. In our analysis of cucumber (*Cucumis sativus*) cutin, we found an unusual cutin component that was not observed in the monomer analysis of our other cutin samples.

RESULTS AND DISCUSSION

8,16-Dihydroxyhexadecanoic acid, **1** [HOOC-$(CH_2)_6$-CHOH-$(CH_2)_7$-CH_2OH] was characterized by interpretation of the mass spectrum of the di-*O*-silyl fatty acid methyl ester derivative {*m/z*, 431 $[M-15]^+$, 289 (scission between C_7 and C_8), 245 (scission between C_8 and C_9)} and the ^1H NMR spectrum was consistent with the structure of 8,16-dihydroxyhexadecanoic acid. The spectrum displayed a 2H triplet at 3.67 ppm for the terminal CH_2OH; a broad 1H multiplet at 3.60 ppm corresponding to the CHOH at position 8; a 2H triplet at 2.35 ppm for the CH_2 at position 2; all other chain methylene resonances were derived in a complex envelope of shifts from 1.23 to 1.70 ppm

Dihydroxy fatty acids are part of the composition of cutin of various plants. A wide range of positional isomers from 7,16- to 10,16-dihydroxyhexadecanoic have been found in various leaf and fruit tissues [4]. Compound **1** has been detected at low levels in grapefruit (*Citrus paradisi* Mac fed.) [5] and apple (*Malus pumila*) [6] cutin. In cucumber cutin, **1** represented more than 70% of the total monomers. The biosynthesis of dihydroxyhexadecanoic acids involved direct hydroxylations of palmitic acid at the *w*- and 9- or 10-positions. The two hydroxylations are catalysed by two different enzymes [7]. The biosynthetic pathway for the formation of **1** could involve a specific hydroxylation in the 8-position of 16-hydroxyhexadecanoic acid.

EXPERIMENTAL

General. ^1H NMR spectra were obtained on a JEOL GX-400 NMR spectrometer operating at 9.3 Tesla. All spectra were recorded in $CDCl_3$ at 25° and shifts are reported in ppm relative to int. TMS. Each spectrum required 128 scans, a 90 pulse width of 7 μsec and a repetition time of 2.8 sec. GC-MS: GC to TMSi esters of hydroxy fatty acids were run with a Hewlett-Packard Model 5990B instrument fitted with an Ultra 1 (methylsilicone) 12 M capillary column temp. programmed from 150 to 250° at 4° min^{-1}. Sepn of hydroxy fatty acids was achieved by HPLC equipped with an evaporative light-scattering detector (ELSD) at 40° and 25 psi of N_2. Columns were (A) a Chrompack ChromSep 7 μm silica column (3.0 × 100 mm) for analytical purposes, (B) a Spherisorb 3 μm silica column (4.6 × 100 mm) for semi-prep. purposes.

Plant material. Discs of peel from fresh cucumber purchased from a local supermarket were removed, boiled in 0.05 M oxalate buffer (pH 4.0) and washed thoroughly with deionized H_2O. Ground cutin was extracted with $CHCl_3$ in a Soxhlet, dried and subsequently treated with a soln of *Aspergillus niger* cellulase and pectinase as previously described [3].

Extraction and analysis. Alkaline hydrolysis was performed by reacting 30 mg of cucumber cutin with 1 ml of

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10% (w/v) KOH in MeOH at 28° for 16 hr. The resulting soln was acidified with HOAc and lipids were extracted with $CHCl_3$–MeOH as previously described [8]. Organic phases were removed and evapd under N_2. The dry residue was weighed and dissolved in 1 ml of $CHCl_3$–MeOH (17:3). The analysis of the resulting cucumber cutin monomers was performed by HPLC (column A, gradient of iso-PrOH with hexane–iso-PrOH–HOAc, 0.5 ml min^{-1}) as previously described [1]. A few mg of 8,16-dihydroxyhexadecanoic acid was collected for NMR spectroscopic analysis using a semi-prep. (column B, gradient of iso-PrOH from hexane–iso-PrOH–HOAc to 20% of iso-PrOH, 1 ml min^{-1}).

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